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(54) Title: SUNFLOWER SEEDS AND OIL HAVING A HIGH STEARIC ACID CONTENT

(57) Abstract

The invention relates to a sunflower seed, comprising a sunflower oil having an increased stearic acid content as compared to wild type seeds, obtainable by treating parent seeds with a mutagenic agent during a period of time and in a concentration sufficient to induce one or more mutations in the genetic trait involved in stearic acid biosynthesis resulting in an increased production of stearic acid, germinating the treated seeds and culturing progeny plants therefrom, collecting and analyzing progeny seeds, selecting seeds that have acquired the desirable genetic trait and optionally repeating the cycle of germination, culturing and collection of seeds. Preferably the seeds comprise an oil having a stearic acid content of between 19.1 and 35 % by weight related to the total amount of fatty acids in the oil, and are obtainable by treating the parent seeds with an alkylating agent, such as ethyl methane sulfonate in water, or with sodium azide in water. The invention further relates to sunflower oil obtainable by extracting the sunflower seeds, to a method for preparing sunflower seeds having an increased stearic acid content as compared to wild type seeds, a method for preparing a sunflower oil having an increased stearic acid content sunflower plants produced from the seeds and the use of the sunflower oil in various products.

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1

SUNFLOWER SEEDS AND OIL HAVING A
HIGH STEARIC ACID CONTENT

The present invention relates to sunflower seeds
5 comprising an oil having an increased stearic acid content
as compared to wild type plants between 10% and 35% by
weight related to the total amount of fatty acids in the
oil. The invention also relates to sunflower seeds having a
stearic acid content up to 54% by weight or more. The
10 invention further relates to a sunflower oil extractable
from the seeds of the invention, to sunflower plants
produced from the seeds, to methods for preparing the seeds
and the oil, as well as to the use of the oil in various
products and to the products comprising the oil.

15 Sunflower is generally cultivated for obtaining
oil which has saturated fatty acids (palmitic and stearic)
and unsaturated fatty acids (oleic and linoleic). The
stearic acid content is always less than 10% (Gustone, F.D.
et al. "The lipid handbook"; Chapman and Hall 1986),
20 normally comprised between 3% and 7%. In relation with the
unsaturated fatty acids there are two different kinds of
sunflower seeds: the normal sunflower which has a linoleic
acid content between 50% and 70% (Knowles, P.F. "Recent
advances in oil crops breeding"; AOCs Proceedings 1988) and
25 the high oleic sunflower which has 2-10% of linoleic acid
and 75-90% of oleic acid (Soldatov, K.I. "Chemical
mutagenesis in sunflower breeding"; Int. Proc. 7th Intern.
Sunflower Conference, 352-357, 1976). There is also a
sunflower line having a high palmitic acid content, between
30 22% and 40% (R. Ivanov et al. "Sunflower Breeding for High
Palmitic Acid Content in the Oil; Proc. of the 12th Intern.
Sunflower Conference, Vol. II, 453-465, 1988) and another
line with low saturated fatty acid content (6% or less) (EP-
A-496504).

35 Table 1 shows the fatty acid composition for some
known sunflower oil varieties.

Table 1

% of fatty acids in sunflower oil

Variety	Palmitic	Stearic	Oleic	Linoleic
Normal ¹	5.9	5.7	21.8	66.5
High oleic ¹	3.1	4.8	84.9	6.7
Low saturated ²	3.9	2.2	89.9	4.0
High palmitic ³	25.1	4.3	10.6	56.4

¹ Fernández Martínez et al.; Grasas y Aceites 37, (1986)² Patent EP-A-496504.³ This variety has also 3.6% of palmitoleic acid

The saturated fatty acid content of an oil is directly related with the physical and chemical characteristics thereof. In case that said content is sufficiently high, the oil can be a solid at room temperature like some animal fats. Normal sunflower oil is always a liquid under said conditions.

In the food industry like for the production of confectionery or margarine, animal fats or hydrogenated vegetable fats are usually used because a solid or semi-solid product is required. By means of hydrogenation unsaturated fatty acids are converted into saturated fatty acids. Animal fats as well as hydrogenated fats are not very recommendable from a nutritional point of view (Chow, C.K. "Fatty acids in food and their health implications", Dekker, N.Y., 1992). Animal fats have a relatively high cholesterol content. Too much cholesterol in the diet may be detrimental to the health. Therefore animal fats have been substituted in the last years by hydrogenated vegetable fats which do not contain cholesterol.

However, said hydrogenated fats present another problem derived from the hydrogenation process. In said process positional isomerization (shift of double bonds) and stereo-chemical transformations (formation of "trans" isomers) take place. Isomers are produced in an amount of up

to 30%-50% of the total fatty acids amount. These isomers are not very healthy from a nutritional point of view (Wood, R., "Biological effects of geometrical and positional isomers of monounsaturated fatty acids in humans"; Dekker, N.Y. (1990); Willet, W.C. & Ascherio, A., "Trans Fatty Acids: Are The Effects Only Marginal ?", American Journal of Public Health, Vol. 84, 5, (1994)). Therefore, the use of hydrogenated fats in the food industry should be avoided.

Sunflower oil has a desirable content of unsaturated fatty acids. For use in the food industry however, the stearic acid content of the oil must be higher than in the normal sunflower oil (Norris, M.E., "Oil substitutions in food formulations", Inform. 1, 388-392 (1990)) in order to obtain a more solid product.

It is thus an object of the invention to provide a new natural vegetable oil extracted from mutated seeds, the oil having a higher stearic acid content as compared to oil obtained from wild type seeds.

The invention therefore provides sunflower seeds, comprising a sunflower oil having an increased stearic acid content as compared to wild type seeds, obtainable by treating parent seeds with a mutagenic agent during a period of time and in a concentration sufficient to induce one or more mutations in the genetic trait involved in stearic acid biosynthesis resulting in an increased production of stearic acid, germinating the treated seeds and culturing progeny plants therefrom, collecting and analyzing progeny seeds, selecting seeds that have acquired the desirable genetic trait and optionally repeating the cycle of germination, culturing and collection of seeds.

Preferably the sunflower seeds according to the invention comprise an oil having a stearic acid content of between 19.1 and 35% by weight, related to the total amount of fatty acids in the oil, and are obtainable by treating the parent seeds during 2 hours at room temperature with an alkylating agent such as a solution of 70 mM ethyl methane sulfonate in water.

In another embodiment of the invention the seeds comprise an oil having a stearic acid content of between 10 and 19% by weight related to the total amount of fatty acids in the oil, and are obtainable by treating the parent seeds with a solution of 2 mM sodium azide in water during 2 hours at room temperature.

Sunflower seeds identified as "CAS-3" having an average stearic acid content of 25% by weight, related to the total amount of fatty acids in the oil, have been deposited on December 14, 1994 with the American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD 20852, U.S.A. under deposit accession number ATCC 75968. Sunflower seeds identified as "CAS-4" having an average stearic acid content of 15.4% by weight, related to the total amount of fatty acids in the oil, have been deposited on the same day with the same institution under deposit accession number ATCC 75969.

Seeds having an even higher stearic acid content between 29 and 54% by weight related to the total amount of fatty acids in the oil, may be obtained according to the invention by crossing sunflowers originating from seeds having a stearic acid content between 19.1 and 35% by weight with sunflowers originating from seeds having a stearic acid content between 10 and 19% by weight, and collecting the seeds.

The invention further relates to sunflower oil having a stearic acid content of between 10 and 54% by weight, preferably between 10 and 35% by weight, related to the total amount of fatty acids in the oil, which may be obtained by extracting sunflower seeds of the invention. Sunflower oil having a stearic acid content of 15.4% by weight related to the total amount of fatty acids in the oil, may be obtained by extracting sunflower seeds having the deposit accession number ATCC 75969. Sunflower oil having a stearic acid content of 25% by weight related to the total amount of fatty acids in the oil, is obtainable by extracting sunflower seeds having the deposit accession number ATCC 75968.

CLAIMS

1. Sunflower seed, comprising a sunflower oil having an increased stearic acid content as compared to wild type seeds, obtainable by treating parent seeds with a mutagenic agent during a period of time and in a concentration sufficient to induce one or more mutations in the genetic trait involved in stearic acid biosynthesis resulting in an increased production of stearic acid, germinating the treated seeds and culturing progeny plants therefrom, collecting and analyzing progeny seeds, selecting seeds that have acquired the desirable genetic trait and optionally repeating the cycle of germination, culturing and collection of seeds.
2. Sunflower seed as claimed in claim 1, characterized in that the seeds comprise an oil having a stearic acid content of between 19.1 and 35% by weight related to the total amount of fatty acids in the oil, and are obtainable by treating the parent seeds with an alkylating agent.
3. Sunflower seeds as claimed in claim 2, characterized in that the parent seeds are treated during 2 hours at room temperature with a solution of 70 mM ethyl methane sulfonate in water.
4. Sunflower seed as claimed in claim 1, characterized in that the seeds comprise an oil having a stearic acid content of between 10 and 19% by weight related to the total amount of fatty acids in the oil, and are obtainable by treating the parent seeds with a solution of 2 mM sodium azide in water.
5. Sunflower seed having a stearic acid content of 25% by weight related to the total amount of fatty acids in the oil, obtainable from the American Type Culture Collection under deposit accession number ATCC 75968.
6. Sunflower seed having a stearic acid content of 15.4% by weight related to the total amount of fatty acids in the oil, obtainable from the American Type Culture Collection under deposit accession number ATCC 75969.

7. Sunflower seed having a stearic acid content between 29 and 54% by weight related to the total amount of fatty acids in the oil, obtainable by crossing sunflowers originating from seeds according to claims 2, 3 and 5 with 5 sunflowers originating from seeds according to claims 4 and 6, and collecting the seeds.

8. Sunflower seed having a stearic acid content as claimed in any one of the claims 1-7, and in addition a palmitic acid content between 3 and 40% by weight or an 10 oleic acid content between 3 and 85% by weight or a linoleic acid content between 2 and 84% by weight, all related to the total amount of fatty acids in the oil, or any combination of one or more of these fatty acid contents, obtainable by crossing plants originating from the mutant seeds according 15 to claims 1-7 with a plant showing a desired phenotype with respect to its fatty acid content.

9. Sunflower oil having a stearic acid content between 10 and 19% by weight related to the total amount of fatty acids in the oil, obtainable by extracting sunflower 20 seeds as claimed in claims 1 and 4.

10. Sunflower oil having a stearic acid content of 15.4% by weight related to the total amount of fatty acids in the oil, obtainable by extracting sunflower seeds as claimed in claim 6.

25 11. Sunflower oil having a stearic acid content between 19.1 and 35% by weight related to the total amount of fatty acids in the oil, obtainable by extracting sunflower seeds as claimed in claims 1, 2 and 3.

12. Sunflower oil having a stearic acid content of 30 25% by weight related to the total amount of fatty acids in the oil, obtainable by extracting sunflower seeds as claimed in claim 5.

13. Sunflower oil having a stearic acid content between 29 and 54% by weight related to the total amount of 35 fatty acids in the oil, obtainable by extracting sunflower seeds as claimed in claim 7.

14. Sunflower oil as claimed in any one of the claims 9-13, further having a palmitic acid content between

3 and 40% by weight, an oleic acid content between 3 and 85% by weight and a linoleic acid content between 2 and 84% by weight, all related to the total amount of fatty acids in the oil, obtainable by extracting sunflower seeds as claimed in claim 8.

15. Method for preparing sunflower seeds having an increased stearic acid content as compared to wild type seeds, by treating parent seeds with a mutagenic agent during a period of time and in a concentration sufficient to induce one or more mutations in the genetic trait involved in stearic acid biosynthesis resulting in an increased production of stearic acid, germinating the parent seeds, culturing progeny plants from the parent seeds, collecting progeny seeds and optionally repeating the cycle of germination, culturing and collection of seeds.

16. Method as claimed in claim 15, characterized in that the parent seeds are treated during 2 hours at room temperature with a solution of an alkylating agent, such as 70 mM ethyl methane sulfonate in water.

17. Method as claimed in claim 15, characterized in that the parent seeds are treated during 2 hours at room temperature with a solution of 2 mM sodium azide in water.

18. Method for preparing a sunflower oil having a stearic acid content of between 10 and 19% by weight related to the total amount of fatty acids in the oil, by extracting sunflower seeds as claimed in claims 1 and 4.

19. Method for preparing a sunflower oil having a stearic acid content of between 19.1 and 35% by weight related to the total amount of fatty acids in the oil, by extracting sunflower seeds as claimed in claims 1, 2 and 3.

20. Method for preparing a sunflower oil having a stearic acid content of between 29 and 54% by weight related to the total amount of fatty acids in the oil, by extracting sunflower seeds as claimed in claim 7.

21. Sunflower plant produced from seeds as claimed in any one of the claims 1-8.

22. Use of a sunflower oil as claimed in claims 9-14 in the production of edible fats or fat mixtures, such as margarine or vegetable-dairy.

23. Use of a sunflower oil as claimed in claims 9-14 in confectionery or bakery.

24. Margarine comprising a sunflower oil as claimed in any one of the claims 9-14.

25. Vegetable-dairy comprising a sunflower oil as claimed in any one of the claims 9-14.

10 26. Confectionery comprising a sunflower oil as claimed in any one of the claims 9-14.

27. Bakery comprising a sunflower oil as claimed in any one of the claims 9-14.

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A01H5/10 A01H1/06

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A01H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP,A,0 496 504 (HEATON) 29 July 1992 cited in the application see claims 1-20 ---	1,9,15, 18,20,21
A	US,A,4 627 192 (FICK) 9 December 1986 see the whole document ---	1,9,15, 18,20,21
A	US,A,4 378 655 (JOHNSON) 5 April 1983 see claims 1-24 ---	15
A	EP,A,0 431 833 (GREEN) 12 June 1991 see page 5, line 3 - line 28 ---	2,3,15, 16
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☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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INTERNATIONAL SEARCH REPORT

ma: 1 Application No
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>PATENT ABSTRACTS OF JAPAN vol. 014 no. 197,23 April 1990 & JP,A,02 039834 (KUMAO KATSUKI) 8 February 1990, see abstract</p> <p>-----</p>	4

INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 95/00369

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EP-A-496504	29-07-92	US-A- 5276264	04-01-94
		AU-A- 1013292	16-07-92
		CA-A- 2058849	10-07-92
		CA-A- 2129621	10-07-92
		HU-A- 66874	30-01-95
		JP-A- 5199821	10-08-93
		NZ-A- 241263	26-10-94

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		CA-A- 2032638	30-05-91
